

A Review of Immunotherapy

PHILIP S. NORMAN

Clinical Immunology Division, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.

Since its introduction in 1911 desensitization or hyposensitization has been practiced by allergists (46) mainly as an art based on clinical observation of individual patients. The lack of quantifiable observation has led many, both inside and outside the specialty, to look at the technique with a questioning eye with the result that a number of critics have doubted the usefulness of desensitization.

Two things are changing this uncomplimentary view. Improved methods for collecting clinical evidence by symptom diaries furnished by patients participating in controlled studies are providing a considerable body of quantitative clinical observation which describes the clinical efficacy of desensitization. Immunologic methods developed in the last 20 years provide data which explain, at least in part, the mechanisms involved.

At the clinical level, distinctions have been made as to which conditions are helped by immunotherapy while some observations have given rough indications of the dose required for amelioration of symptoms. In 1955, Frankland & Augustin carried out the first placebo-controlled trial of desensitization employing both crude and partially purified grass pollen extracts in patients with hay fever and hay asthma. Both the rhinitis and asthma were relieved by either type of extract in a fashion that met appropriate statistical tests of significance (17). Johnstone obtained similar results in a placebo-controlled study of ragweed sensitive children with autumn rhinitis and asthma.

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Low dose treatment was ineffective (27). Lowell & Franklin carried out two carefully performed studies in ragweed sensitive adults with autumn hay fever and found that the addition of ragweed extract to a treatment regimen produced a significant reduction in rhinitis in the August-September season. Their evidence indicated that the results of treatment were specific and that treatment with other allergens did not control ragweed-induced symptoms (39, 40).

Studies performed in our clinic over a number of years with ragweed-sensitive patients with hay fever showed that the efficacy of preseasonal courses of injections of ragweed extract depended on the dosage administered. Low dosage courses were relatively ineffective whereas high doses were more regularly effective (35, 36, 37, 47, 49). Because of standardization problems (to be discussed later) the optimum dose is at this date still difficult to describe, but it appears that a dose of crude unaltered extract adequate to insure symptomatic relief will often be accompanied by a considerable risk of large local or systemic allergic reactions. The only other pollen studied is mountain cedar. Recently, a carefully performed study shows that immunization controls seasonal symptoms due to this pollen occurring in the Southwestern United States (52).

In housedust sensitivity results are mixed. Some of the conflicting results may arise out of differences in dosage. For instance, in housedust-related asthma a cooperative study of treatment with housedust extract in rather modest doses revealed no relief after 15 weekly injections (15), whereas a study by Aas of housedust-sensitive children treated

with housedust extract for 2½ years to 3 years in large doses showed not only symptomatic improvement in comparison with controls but also a reduction in reactivity upon bronchial challenge (1). This difference may be explained by the observation of May et al. that unusually large amounts of housedust extracts (in terms of protein nitrogen) are necessary in order to obtain a blocking antibody response (42). It appears likely that the large doses employed over a long period by Aas were necessary to demonstrate efficacy. Subsequently, a study with housedust mite extract has demonstrated amelioration of housedust symptoms in an area where housedust mites seem to be a major factor in housedust allergy (60). In contrast, bacterial vaccines in a number of controlled studies have consistently failed to be efficacious in the treatment of asthma related to respiratory infections (2, 6, 14, 18, 21, 28). These findings are supported by more recent studies indicating that respiratory infections in asthmatics are usually initiated by the same viruses that afflict the general population and are uncommonly associated with bacteria (22, 45).

Placebo-controlled studies with other pollens, molds and animal danders are completely lacking. Indeed, the variety of allergenic substances in the environment that may at times cause symptoms is so great as to make it improbable that studies which require so much labor will be performed even for the more important allergens. It therefore seems appropriate to try to understand immunologic and physiologic mechanisms responsible for the results of immunotherapy in the hope that the principles learned will allow evaluation of efficacy by techniques simpler than full-scale clinical study.

Immunologic studies appear to point the way to this goal although final answers have not yet been obtained. Most of these studies have been performed employing ragweed allergy as a model disease. This is partly because ragweed allergy is common in the United States, affecting perhaps 5% of the

population and partly because the principal allergens of ragweed have been isolated providing useful tools for research. The principal allergen of ragweed (antigen E) is a simple globular protein with a molecular weight of 38,000. It represents 0.5% of the water extractable solids of ragweed pollen and 6% of the protein. It is highly active in allergic individuals, often requiring a concentration of no more than 0.00001 µg/ml to induce a positive skin reaction by the intradermal route (29). Further studies showed that similar concentrations would induce populations of isolated washed leukocytes from allergic patients to release histamine *in vitro*. The concentration required for a 2+ intradermal skin test or for 50% histamine release varies greatly from one allergic person to another and correlates with the severity of hay fever symptoms suffered during natural exposure as reported by symptom diaries (37). Histamine release from leukocytes provides more precise quantitative data than skin tests and has, therefore, been used to predict severity of symptoms with some success. For this reason it may be used as a basis for selecting matched groups of patients in whom comparisons can be made between several alternative methods of treatment, thus more readily providing quantitative data (36).

Furthermore, the technique can be turned to the measurement of serum antibodies which block the allergic reaction. Blocking antibodies were first noted after immunotherapy by Cooke et al. who demonstrated that serum from treated individuals blocked the passive transfer of reagin in skin (9). The more sensitive and precise histamine-release technique showed that every allergic individual has a small amount of blocking antibody in serum and that injections raise the amount of antibody in a dose-related fashion. In treated groups of patients the relationship between severity of symptoms and leukocyte histamine release is altered but not in a way that can be predicted by measurement of blocking antibody (37). Nor can the degree of

symptom relief be accurately predicted by blocking antibody titer. Nevertheless, there is some relationship between symptoms and antibody titers. In a group of patients undergoing immunotherapy, the half with the greater antibody response suffered less severe symptoms during seasonal exposure to pollen than the half with the lesser antibody response (35).

Before the relationship between blocking antibodies and the result of treatment can be discussed fully other immunologic changes must be considered. Pruzansky & Patterson noted that high-dosage immunotherapy could render the leukocytes of ragweed-sensitive individuals completely unreactive to ragweed extract *in vitro*, histamine release becoming essentially zero (55). Subsequently, it was shown that there are two phases in the development of this change as treatment progresses. First, there is a change in "cell sensitivity" in that a higher concentration of antigen is required for histamine release. This is sometimes followed by a change in "cell reactivity" in that at optimum concentration of antigen less than the full cellular store of histamine is released. In the final stage, which only a few treated patients develop, virtually no histamine release occurs at any concentration. In studies in children, although patients who developed this change more often had good relief of symptoms, they were not invariably relieved; hence, development of less reactive cells had little value in predicting the result of treatment (58). Subsequently, Lichtenstein & Levy noted that patients who lost cellular reactivity to ragweed after treatment with ragweed invariably failed to show cellular reactivity to other antigens. They suggested that this change was therefore not immunologically specific and represented a general loss of reactivity (33). To test this hypothesis a study in our clinic to examine the specificity of treatment was organized employing patients sensitive to both grass and ragweed pollens. Immunization with ragweed pollen extract in half the patients produced a significant amelioration of all

ragweed symptoms when compared with the untreated half of the patients. Symptoms during the grass season were identical in the two halves, so that no nonspecific effect was demonstrated. It so happened that none of the treated patients had a reduction in leukocyte sensitivity of reactivity so that the possibility that a nonspecific mechanism for improvement may at times be at work remained open. The greater part of the results of treatment seemed, however, to be specific.

The discovery that reaginic antibodies are of the IgE class which fix to the surface of basophiles and mast cells sensitizing them for release, not only of histamine but also mediators such as SRS-A and ECF-A, has given the study of these antibodies a central place in understanding allergy (23). The discovery of IgE antibodies has also led to the preparation of highly specific anti-IgE antibodies raised in animals. Radiolabeled anti-IgE can be used to quantify specific IgE antibodies reacting with antigens coupled to insoluble carriers, a method called RAST (radioallergosorbent technique) (64). For the first time, RAST provides a satisfactory way to measure the extremely small amounts of IgE antibodies in serum. Using the laborious technique of passive transfer of reagins to isolated washed leukocytes *in vitro*, Levy & Osler had shown that immunotherapy reduced the seasonal rise of ragweed reagins during and following pollen exposure (31). Employing RAST, our group followed IgE antibodies to ragweed over several years in both treated and untreated patients. In untreated patients each seasonal exposure resulted in a rise of IgE antibodies during and immediately after the season. Titers fell slowly during the subsequent winter, spring and summer but never to undetectable levels. In patients receiving perennial treatment the seasonal rise was either reduced or ablated. As the off-season fall in titer continued, the titer was distinctly lower than the pretreatment titer after two seasons of observation in 20 of 21 patients. On the other hand, titers were still detectable at a level sufficient to

sensitize leukocytes, so that it was difficult to attribute treatment results to the modest reduction in IgE antibody titer. An important point perhaps is that the patients who had the greatest rise in IgG blocking antibodies had the greatest reduction in IgE antibodies (32). As these changes were not related temporally, it is difficult to ascribe a cause-and-effect relationship. Furthermore, a series of experiments in mice suggest a different mechanism; i.e., stimulation of specific suppressor T cells. King et al. found that treatment of antigen E with denaturing agents (such as urea) separates two polypeptide chains, alpha and beta, linked by noncovalent forces. The antigenic determinants of antigen E depend on the maintenance of the linkage between the two chains, hence the individual chains or mixture of the separated chains are poorly antigenic and stimulate little antibody response (30). The chains, however, retain the ability to stimulate either helper T cells or suppressor T cells, depending on circumstances. Virgin mice treated with a mixture of separated chains in the form of urea denatured antigen E (UDE) show helper T-cell activity when adoptive transfers of lymphocytes are given to mice immunized with native antigen E. On the other hand, mice immunized with antigen E and producing IgE antibody will show partial suppression of IgE synthesis when treated with UDE. Adoptive transfer experiments show the presence of IgE-specific suppressor T cells (24).

It seems a reasonable hypothesis that treatment of allergic humans with ragweed extract stimulates suppressor T-cell activity, thus reducing IgE synthesis specifically. At this time, there is no way of testing for specific suppressor T-cell activity in man so that direct study of the hypothesis is not possible. Several measures of lymphocyte function, however, are altered during immunotherapy. Patients allergic to ragweed frequently show tritiated thymidine incorporation, mitogenic factor (MF) production, or macrophage inhibition factor (MIF)

production by peripheral blood lymphocytes *in vitro* in the presence of antigen E or other ragweed extracts (57). After a course of immunotherapy these measures are usually reduced (12).

Specific stimulation of suppressor T cells for IgE synthesis by nonantigenic materials has distinct attractions as a goal for treatment of allergic reactions. Nonantigenic materials would have little risk of untoward allergic reactions, while specific suppression of undesirable IgE synthesis would leave other potentially desirable IgE functions intact.

In our clinic a course of treatment with UDE was tried in eight ragweed-sensitive patients. An initial preseasonal course showed that the material was safe to administer but gave little evidence of IgE antiragweed suppression. A second perennial course in larger doses did show evidence of suppression of the seasonal rise in IgE antiragweed, particularly in the patients who received the highest doses. There was no rise in IgG antibodies to ragweed but there was reduction in tritiated thymidine incorporation in the presence of antigen E in some of the patients (not necessarily the ones who showed suppression of IgE synthesis). The degree of suppression of IgE was, however, not clinically useful, as the patients had their usual hay fever symptoms. A practical regimen for specific suppression of IgE synthesis still remains to be achieved.

Antibodies in secretions are also stimulated by immunotherapy. Although an early report from our laboratory suggested that nearly everyone in the United States had nasal secretory IgA antibody to two allergens common in the United States, ragweed and grass (62), subsequent study employing better methods showed this conclusion to be incorrect. Double antibody radioimmunoassays specific for IgG and IgA antibodies to ragweed show that nonallergic individuals rarely have ragweed antibodies in nasal secretions, whereas allergic individuals regularly have small amounts of antibodies of both classes (53). Individuals undergoing

immunotherapy regularly showed increased amounts of both antibodies. As the ratio of IgG to secretory IgA was unchanged after immunotherapy, IgA antibody was absent in serum, and the IgG antibody titers in secretions had no relationship to IgG titers in serum, it was suggested that both classes of antibody were synthesized locally (53).

Both classes of antibody were capable of blocking histamine release and their appearance in secretions would appear to be an ideal first line of defense against pollens impinging on mucous membranes. This idea is particularly attractive because recent studies have shown that secretions contain populations of living mast cells sensitized with IgE to release mediators upon contact with allergens to which the donor is sensitive. In the study of secretional antibodies in treated patients, the number of patients studied was too small to attempt any correlation with result of treatment.

The immunologic changes induced by immunotherapy may be summarized as follows:

1. Serum IgG antibodies are increased
2. IgG and secretory IgA antibodies in secretions are increased
3. IgE antibodies in serum are reduced but not eliminated
4. Several measures of lymphocyte function in the presence of specific antigen are reduced
5. Mediator containing cells at times shows reduced sensitivity or reactivity.

It seems unlikely that any one of these changes accounts entirely for the results of immunotherapy, but that a complicated interplay of these, and perhaps other undiscovered, factors may be at work. Some relative importance may be guessed at, however. The degree of suppression of IgE synthesis seems unlikely to be useful at least in the first year or two of treatment. This leaves open the possibility that many years of treatment suppresses IgE production more completely. A study by Connell et al. of

patients treated for many years showing virtual absence of skin-sensitizing antibody raises this possibility (8).

Changes in leukocyte sensitivity or reactivity do not occur with any regularity in adults but appear more frequently in children. Whether this finding will account for the apparently greater success of immunotherapy in children remains a matter for speculation. In any event, significant amelioration of symptoms can occur in the absence of any change in leukocyte reactivity. The increases in secretional antibodies observed in the small number of patients studied stimulate speculation, but more patients will need to be studied before a correlation with clinical results can be attempted. This leaves increase in serum IgG antibodies as the major immunologic change to consider. This is the only change to date to show significant, albeit rough, correlation with symptom scores. In considering improved methods it seems reasonable to concentrate on those regimens or preparations which produce the maximum antibody response. Methods which raise serum IgG antibodies seem likely to lower IgE antibodies also. A first step in this direction is simply to insure that extracts furnished to physicians contain the relevant antigens in concentrations adequate to stimulate immunologic responses. It is readily apparent that commercially prepared extracts standardized by PNU or weight-by-volume methods do not regularly attain this simple goal. Ragweed extracts from different concerns with the same label varied one hundredfold in their content of antigen E and biological activity in inciting skin tests or leukocyte histamine release. Even two lots from the same company showed a one hundredfold difference (4). Grass extracts also showed considerable but less variation (5). It has already been noted that several dust extracts had a low capability for inciting IgG responses. Commercial extracts of *Alternaria* appear to have miniscule amounts of the principal allergenic protein of this mold (65).

There are several approaches to assuring the quality of allergen extracts. One requires identification and isolation of the allergenic proteins of the crude allergen. Precipitating antisera can then be raised in animals with this isolated allergen and unknown extracts can then be assayed for allergen content by double diffusion in gel. This has been done with antigen E of ragweed and Group I of rye grass. Fortunately, the principal allergens of a number of species of grass crossreact so that antisera raised to rye Group I can be used to standardize extracts from these species (5). Although the allergens in a number of materials have been identified, the number of allergens not studied is vastly greater and it seems impractical to standardize all of them by this laborious method.

RAST inhibition employing serum pools from patients with specific allergies allows comparative assays of the mixture of allergens in crude extracts and may successfully by-pass the isolation procedure. Whether RAST inhibition will be sufficiently quantitative and universally applicable requires further testing (16). Crossed radio-immuno-electrophoresis employing I^{125} -labeled anti-IgE identifies allergens without prior isolation and can be used to quantify a number of allergens in a crude extract. It appears to provide the sophistication required for a definitive analysis of numerous allergens (63).

Even if potent extracts were regularly available, it is not evident that the regimens commonly used by allergists are those which will produce optimum antibody responses. This subject has been little studied and needs attention. Assessing treatment regimens by observing clinical results is probably too tedious to perform in a systematic fashion but can more easily be done immunologically, as IgG antibody responses are readily measurable by double antibody radioimmunoassay and IgE antibodies by RAST.

Considerable effort is being expended in modifying extracts in ways that will slow

absorption, increase antigenicity, or reduce allergenicity. One of the earliest techniques for slowing absorption is simple precipitation with alum as performed with several vaccines and toxoids. Fairly extensive study of this method has been performed with ragweed. Antigen E is largely precipitated from crude extracts by the method. Successful preseasonal immunization can be carried out in about half as many injections as with conventional aqueous extracts. IgG antibody responses are similar to those obtained with similar doses of aqueous extract, so that the principal advantage appears to lie in slower absorption which allows a larger dose in each injection and, consequently, fewer injections. This advantage still holds for subsequent years of retreatment in preseasonal courses where fewer injections are needed for an adequate dose. Efficacy is as good as the aqueous extract but no better. Alum-precipitated extracts represent a modest advance which is currently available to physicians (50).

Mineral oil is a more effective immunologic adjuvant than alum in animal work. Trials in humans were reported enthusiastically at first (7, 13, 38) but controlled trials showed at best modest efficacy (3, 10, 11, 19, 48, 59). Local nodules and sterile abscesses were a problem; moreover, mineral oil is not metabolized and remains in the tissues indefinitely. Plasma cell tumors develop in certain strains of mice after intraperitoneal injections of mineral oil, creating a fear of carcinogenesis in man (54).

Another modified material combines extraction with the organic solvent pyridine followed by alum-precipitation (20). Although allergens so prepared give a low rate of untoward reactions, pyridine extraction has variable effects on allergens. Antigen E of ragweed, for instance, is almost entirely denatured (34). While antibody responses to ragweed have been detected in treated patients (56, 61), no comparisons with responses to other methods of allergen preparation are available.

Marsh et al. developed an "allergoid" by

treating rye grass allergen with formaldehyde under mild conditions. A cross linkage between molecules was produced which reduces allergenicity (ability to elicit skin tests or histamine release) while maintaining antigenicity (ability to stimulate IgG antibody synthesis) (41). Preliminary trials in ragweed-sensitive patients with ragweed allergoid show that it is safe to administer and produces good antibody responses. Whether the usual course of immunization can be shortened is now under study.

Glutaraldehyde may also be used to produce aggregates or polymers. Such polymers are more slowly adsorbed after injection than native allergens and produce somewhat greater antibody responses. An early trial has shown good efficacy in ragweed hay fever (43, 51).

Allergens have also been treated with glutaraldehyde and adsorbed to suspensions of L-tyrosine (44). With some allergens the incidence of systemic reactions has been reduced to the point where treatment has been accomplished in three injections. Antibody responses have been favorable but comparisons with standard methods are not available at this writing (25, 26).

In summary, controlled observations have confirmed many but not all empirical observations on the clinical effects of immunization with allergenic extracts. Immunologic methods provide valuable but as yet incomplete clues as to the mechanisms of improvement after immunization. Such observations have the potential for leading to improvements in immunotherapy which may shorten the course of immunization, eliminate allergic side reactions or improve efficacy.

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Address:

Philip S. Norman, M.D.

The Johns Hopkins University
School of Medicine at
The Good Samaritan Hospital
5601 Loch Raven Boulevard
Baltimore, MD 21239 U.S.A.

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